NAD- AND NADP-DEPENDENT DEHYDROGENASE ACTIVITIES IN ERYTHROCYTES UNDER EXPERIMENTAL HYPOXIA

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16. Abstract The activity of some NAD- and NADP-dependent enzymes in rat erythrocytes was studied under hypoxid hypoxia and adrenal myocarditis. It is shown that under acute hypoxic hypoxia the activity of glucose-6-phosphate-dehydrogenase in a nucleus-free erythrocyte is inhibited reversibly, the glutathione reductase activity immediately connected with the former being unchanged. The activity of lactate dehydrogenase increased. Under a considerable dose of adrenalin, the activity of glutathione reductase rose. Thus, under acute hypoxia, the ratio between the aerobic and anaerobic pathways of energy formation in the erythrocyte is disturbed in favor of the anaerobic pathway.

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ANNOTATION

The activities of some NAD- and NADP-dependent enzymes of rat erythrocytes were studied in hypoxic hypoxia and adrenal myocarditis. It was shown that, in acute hypoxic hypoxia, activity of glucose-6-phosphate-dehydrogenase (D-glucose-6-phosphate: NADP-oxidoreductase, E.C. 1.1.1.49) is reversibly inhibited in nucleus-free erythrocytes; the glutathione reductase (reduced NAD(P): oxidized glutathione-oxidoreductase, E.C. 1.6.4.2) activity directly associated with it does not decrease, while the activity of lactate dehydrogenase (L-lactate: NAD-oxidoreductase, E.C. 1.1.1.27) increases.

As a result of a considerable dose of adrenalin, the activity of glutathione reductase in the erythrocytes increases, while the glucose-6-phosphate-dehydrogenase activity decreases.

Thus, under extreme conditions, the ratio between the aerobic and anaerobic pathways of energy formation in the erythrocyte is disturbed, in favor of the anaerobic pathway.

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The activity of some NAD- and NADP-dependent enzymes in rat erythrocytes was studied experimentally under hypoxic hypoxia and adrenal myocarditis. Experiments were conducted with white male rats weighing 150 to 200 g which were divided into three groups; 1) control; 2) animals under the influence of hypoxic hypoxia, and 3) rats subjected to experimental myocarditis. The hypoxic hypoxia was caused by keeping the animals for 60 min in a pressure chamber at 7500 m simulated altitude, and acute adrenal myocarditis was created by Vishnevs'kaya's technique [Bull. eksperi. biol. i medit. 10, 29, 1954]. It was established that during acute hypoxic hypoxia the activity of glucose-6-phosphate-dehydrogenase in the cell of the nucleus-free erythrocyte is inhibited reversibly; the glutathione reductase activity directly associated with it does not change, while the activity of lactate dehydrogenase increases. Under the effect of a considerable dose of adrenalin, the activity of glutathione reductase increases. Thus, under the influence of acute hypoxia, the ratio between the aerobic and anaerobic ways of energy formation in the erythrocyte is disturbed in favor of the anaerobic way.

NAD- AND NADP-DEPENDENT DEHYDROGENASE ACTIVITIES IN ERYTHROCYTES UNDER EXPERIMENTAL HYPOXIA

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We demonstrated earlier that, because of acute hypoxia, the /564** ratio of the oxidized and reduced forms of nicotinamide coenzymes is decreased [1], which leads to a decrease in the activity of types of dehydrogenases dependent on them [2, 3].

In this work, we studied cells with a simpler organization, nucleus-free erythrocytes, in which there are a number of singularities in metabolism. Besides, in these cells, the role of processes connected with NAD and NADP is most important, since these reactions, to a great extent, fix the energy formation and, consequently, protect the integrity of the cells. Data in the literature in the main fits metabolism in the erythrocytes, in adaptation to hypoxia [4]. We studied the effect of extreme stages, hypoxic hypoxia and acute adrenalmyocarditis, on the activities of the enzymes mentioned above, connected with NAD and NADP.

Procedure

The investigations were carried out on white male rats, weighing 150-200 g, divided into three groups: The first, the control animals; the second, those undergoing hypoxic hypoxia; and the third, rats, in which experimental myocarditis was induced.

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Hypoxic hypoxia was caused, by means of subjecting the animals to a simulated altitude of 7500 m in a barochamber, for a period of 60 min. The animals were studied in the first 5 min and 60 min after the "descent." Acute adrenalmyocarditis was induced as described by Vishnevs'kaya [5]. Dehydrogenase activity was determined spectrophotometrically, in terms of the linear dependence of activity of the enzyme with several proteins in a time test [6-8].

Hemoglobin in the lysates was determined spectrophotometrically, by absorption at 540 nm, and the lactate dehydrogenase isoenzymes in the lysates (1:5 dilution) were studied by electrophoresis on agar, as we described earlier [3]. The method of comparison of the totals of paired versions was used for statistical processing of the data obtained [9].

Results and Discussion /565

In acute hypoxic hypoxia, a reduction in the activity of the most important enzyme of the pentose phosphate pathway, glucose-6-phosphate-dehydrogenase is distinguished, as it specifies the occurrence of aerobic processes in the erythrocytes (Table 1).

Data in the literature indicate that a natural deficit of this enzyme is accompanied by symptoms of hemolytic anemia [10, 11]. Thus, this enzyme has an important part in preserving the integrity of the erythrocyte. Our observations showed that this reduction in enzyme activity is reversible. In animals which lived for a period of 1 year after the "descent," under ordinary conditions, the activity of this enzyme was restored to normal. It is possible

that, in hypoxia, the enzyme undergoes a certain allosteric transformation, not leaving the cell, which is evidence of the stability of its activity in the blood serum.

Glucose-6-phosphate-dehydrogenase functionally is closely interrelated with glutathione reductase activity. The latter supports restoration of the state of glutathione, by way of oxidation of $NADP \cdot H_2$, which is formed, in particular, as a consequence of dehydrogenation of glucose-6-phosphate. According to the data of Bush and colleagues [12], the glutathione-S-S:glutathione-SH ratio normally is 1:99. Support of the renewed glutathione, in turn, is necessary for protection of the SH group of the active center of glucose-6-phosphate-dehydrogenase.

According to our data (Table 2), glutathione reductase activity is not reduced in acute hypoxia.

In acute myocarditis, which is accompanied by hypoxia of hemo- /566
dynamic origin, glucose-6-phosphate-dehydrogenase activity is reduced a little. The possibility is not excluded that the enzyme has a direct effect on adrenalin by the products of its oxidation.

Glutathione reductase activity certainly increases in adrenalmyocarditis. This can be regarded as a compensatory reaction, which stipulates an augmentation of the regenerated glutathione in the cell and, consequently, protecting the active centers of the thiol enzymes, glucose-6-phosphate-dehydrogenase, in particular.

There are data, which are evidence that the activity of glutathione reductase depends on FAD or FMN [13]. It can be granted that, under extreme conditions, the flavin enzymes are less sensi-

TABLE 1

ACTIVITY OF GLUCOSE-6-PHOSPHATE-DEHYDROGENASE OF ERYTHROCYTES (in M per g Hb per min) AND BLOOD SERUM OF RATS ($\Delta\varepsilon_{340}$ per min per ml) IN ACUTE HYPOXIC HYPOXIA

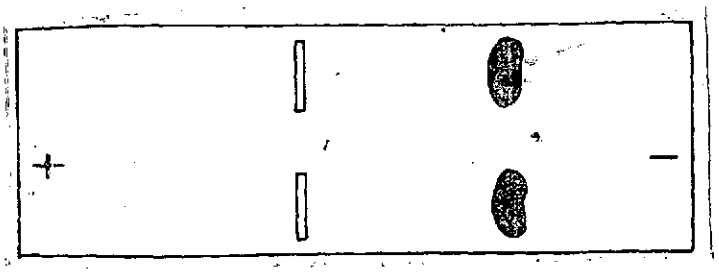
Object of Study	Statistical Indicator	Standard	Hypoxia	
			5 min after "descent"	60 min after "descent"
Erythrocytes	$M \pm m$	2,0±0,2	1,4±0,2	1,7±0,1
	n	15	10	5
	p		$<0,05$	$>0,05$
Blood Serum	$M \pm m$	86±5	94±9	
	n	6	6	
	p		$>0,05$	

Note: The values of p in the Table were calculated in comparison to the standard.

TABLE 2

ACTIVITY OF DEHYDROGENASE BOUND TO NAD OR NADP IN ERYTHROCYTES (in M per g Hb per min) IN EXPERIMENTAL HYPOXIA

Enzyme	Statistical Indicator	Standard	Hypoxic Hypoxia	Adrenal Myocarditis
Glutathione Reductase	$M \pm m$	0.42±0.03	0.50±0.08	0.65±0,14
	n	8	7	8
	p		>0.05	<0.05
Lactate Dehydrogenase	$M \pm m$	5,7±1,0	8,2±0,5	6,7±1,1
	n	10	5	10
	p		$<0,05$	>0.05



LDH-5 Isoenzyme in normal erythrocyte (upper) and in hypoxia.

tive than NADP-dependent dehydrogenases.

The activity of lactate dehydrogenases quite measurably reflects the state of anaerobic processes in the erythrocytes. The lactate dehydrogenase reaction is the basic reaction of oxidation of regenerated NAD. In case of acute hypoxia, there is certainly an increase in activity of this enzyme.

The isoenzyme spectrum of lactate dehydrogenases in erythrocytes is represented by a single LDH-5 cathode fraction (see figure), which does not indicate a transformation in acute hypoxia. Concerning the absence of a genetic apparatus in the cell being studied, it might be supposed that, under these conditions, the enzyme will be activated.

In this manner, in cells with the simplest organization, i. erythrocytes, under extreme conditions, the metabolism will be transformed, by activation of the anaerobic energy formation pathway, which assists in the maximum adaptation of the erythrocytes to the new conditions and is directed towards protection of the integrity of the cell.

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